MAYO CLINIC LABORATORIES

# Test Definition: BTKSG

Bruton Tyrosine Kinase, BTK Full Gene Analysis, Varies

## Overview

### Useful For

Confirming a diagnosis of X-linked agammaglobulinemia in patients with a history of recurrent sinopulmonary infections, profound hypogammaglobulinemia, and less than 1% peripheral B cells, with or without abnormal Bruton tyrosine kinase (BTK) protein expression by flow cytometry

Evaluating for the presence of *BTK* variants in family members of affected individuals, including those who do not demonstrate carrier phenotype by BTK flow cytometry

### Reflex Tests

| Test Id | Reporting Name | Available Separately | Always Performed |
|---|---|---|---|
| CULFB | Fibroblast Culture for Genetic Test | Yes | No |

### Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in the *BTK* gene associated with X-linked agammaglobulinemia.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, recurrence risk assessment, familial screening, and genetic counseling for X-linked agammaglobulinemia.

### Testing Algorithm

For skin biopsy or cultured fibroblast specimens, fibroblast culture will be performed at an additional charge. If viable cells are not obtained, the client will be notified.

### Special Instructions

- Informed Consent for Genetic Testing
- Bruton Tyrosine Kinase (BTK) Genotype Patient Information
- Informed Consent for Genetic Testing (Spanish)

### Method Name

Sequence Capture and Targeted Next-Generation Sequencing (NGS) followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing

### NY State Available

Yes

## Specimen

## Specimen Type
Varies

## Ordering Guidance
Targeted testing for familial variants (also called site-specific or known variants testing) is available for variants identified in the BTK gene. See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about testing option, call 800-533-1710.

## Additional Testing Requirements
To confirm a diagnosis of X-linked agammaglobulinemia in male patients, the preferred approach is to order this test concurrently with BTK / Bruton Tyrosine Kinase, Protein Expression, Flow Cytometry, Blood.

## Shipping Instructions
Specimen preferred to arrive within 96 hours of collection.

## Necessary Information
Bruton Tyrosine Kinase (BTK) Gene Sequencing Patient Information form (T620) is highly recommended. Testing may proceed without the patient information. However, it aids in providing a more thorough interpretation. Ordering providers are strongly encouraged to complete the form and send it with the specimen.

## Specimen Required
**Patient Preparation:** A previous bone marrow transplant from an allogenic donor will interfere with testing. Call 800-533-1710 for instructions for testing patients who have received a bone marrow transplant.

**Submit only 1 of the following specimens:**

**Specimen Type:** Whole blood
**Container/Tube:**
**Preferred:** Lavender top (EDTA) or yellow top (ACD)
**Acceptable:** Any anticoagulant
**Specimen Volume:** 3 mL
**Collection Instructions:**
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**
**Specimen Stability Information:** Ambient (preferred) 4 days/Refrigerated

**Specimen Type**: Skin biopsy
**Supplies**: Fibroblast Biopsy Transport Media (T115)
**Container/Tube**: Sterile container with any standard cell culture media (eg, minimal essential media, RPMI 1640). The solution should be supplemented with 1% penicillin and streptomycin.
**Specimen Volume**: 4-mm punch
**Specimen Stability Information**: Refrigerated (preferred)/Ambient
**Additional Information:** A separate culture charge will be assessed under CULFB /Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

**Specimen Type**: Cultured fibroblasts
**Container/Tube**: T-25 flask
**Specimen Volume**: 2 Flasks
**Collection Instructions**: Submit confluent cultured fibroblast cells from a skin biopsy from another laboratory. Cultured cells from a prenatal specimen will not be accepted.
**Specimen Stability Information**: Ambient (preferred)/Refrigerated (<24 hours)
**Additional Information:** A separate culture charge will be assessed under CULFB /Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

## Forms

1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing (Spanish) (T826)
2. Bruton Tyrosine Kinase (BTK) Gene Sequencing Patient Information form (T620)

## Specimen Minimum Volume

Blood: 1 mL; Skin biopsy or cultured fibroblasts: See Specimen Required

## Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

## Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Varies | Varies | | |

## Clinical & Interpretive

### Clinical Information

X-linked agammaglobulinemia (XLA) is a humoral primary immunodeficiency affecting male patients in approximately 1 in 200,000 live births. XLA is caused by variants in the Bruton tyrosine kinase gene (*BTK*), which results in a profound block in B-cell development within the bone marrow and a significant reduction, or complete absence, of mature B cells in peripheral blood.(1,2)

Approximately 85% of male patients with defects in early B-cell development have XLA. Due to the lack of mature B cells, XLA patients have markedly reduced levels of all major classes of immunoglobulins in the serum and are, therefore, susceptible to severe and recurrent bacterial infections.(2) Pneumonia, otitis media, enteritis, and recurrent sinopulmonary infections are among the key diagnostic clinical characteristics of the disease. The spectrum of infectious complications also includes enteroviral meningitis, septic arthritis, cellulitis, and empyema, among others. XLA typically manifests in male infants.(2) However, other patients present with milder phenotypes, resulting in diagnosis later in childhood or in adulthood. Delayed diagnoses can be partly explained by the variable severity of XLA, even within families in which the same variant is present. X-inactivation of this gene is not typical, and XLA in female patients has

rarely been reported.(3) Therefore, female patients with clinical features that are identical to XLA should be first evaluated for autosomal recessive agammaglobulinemia and for XLA if their biological father is affected with the disease.

A diagnosis of XLA should be suspected in male patients with early-onset bacterial infections, marked reduction in all classes of serum immunoglobulins, and absent B cells (CD19+ cells). The decrease in numbers of peripheral B cells is a key feature, although this can also be seen in a small subset of patients with common variable immunodeficiency. Conversely, some *BTK* variants can preserve small numbers of circulating B cells and, therefore, all 3 of the criteria mentioned above need to be evaluated.(2)

The preferred approach for confirming a diagnosis of XLA in male patients and identifying female carriers requires testing for the BTK protein expression on B cells by flow cytometry and genetic testing for a *BTK* variant. Patients can be screened for the presence of BTK protein by flow cytometry (BTK / Bruton Tyrosine Kinase [BTK], Protein Expression, Flow Cytometry, Blood); however, normal results by flow cytometry do not rule out the presence of a *BTK* variant with normal protein expression but aberrant protein function. The diagnosis is confirmed only in those individuals with appropriate clinical history who have a disease-causing variant identified within *BTK* by gene sequencing or who have male family members with hypogammaglobulinemia with absent or low B cells.

## Reference Values

An interpretive report will be provided.

## Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(4) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

## Cautions

Bruton tyrosine kinase (BTK) protein and genetic tests are not meant for patients with hematological neoplasias on kinase inhibitor therapy, including, but not restricted to, the selective BTK inhibitor, Ibrutinib. This test is meant for the assessment of patients with a suspected monogenic primary immunodeficiency, X-linked agammaglobulinemia, caused by germline variants in the *BTK* gene.

Clinical Correlations:
Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of a reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact Mayo Clinic Laboratories genetic counselors at 800-533-1710.

Technical Limitations:
Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the

minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions (delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

Deletion/Duplication Analysis:
This analysis targets single and multi-exon deletions/duplications; however, in some instances, single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.

For detailed information regarding gene specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent non-leukoreduced blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

Reclassification of Variants**:**
Currently, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages health care providers to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time. Due to broadening genetic knowledge, it is possible that the laboratory may discover new information of relevance to the patient. Should that occur, the laboratory may issue an amended report.

Variant Evaluation:
Evaluation and categorization of variants are performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline.(4) Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and periodic updates to

these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. These findings will be carefully reviewed to determine whether they will be reported.

### Clinical Reference

1. Tsukada S, Saffran DC, Rawlings DJ, et al: Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. Cell. 1993 Jan 29;72(2):279-290
2. El-Sayed ZA, Abramova I, Aldave JC, et al: X-linked agammaglobulinemia (XLA):Phenotype, diagnosis, and therapeutic challenges around the world. World Allergy Organ J. 2019;12(3):100018. doi: 10.1016/j.waojou.2019.100018
3. Takada H, Kanegane H, Nomura A, et al: Female agammaglobulinemia due to the Bruton tyrosine kinase deficiency caused by extremely skewed X-chromosome inactivation. Blood. 2004 Jan 1;103(1):185-187
4. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee: Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015 May;17(5):405-424
5. Valiaho J, Smith CI, Vihinen M: BTKbase: the mutation database for X-linked agammaglobulinemia. Hum Mutat. 2006 Dec;27(12):1209-1217
6. Lopez-Granados E, Perez de Diego R, Ferreira Cerdan A, Fontan Casariego G, Garcia Rodríguez MC. A genotype-phenotype correlation study in a group of 54 patients with X-linked agammaglobulinemia. J Allergy Clin Immunol. 2005 Sep;116(3):690-697. doi: 10.1016/j.jaci.2005.04.043

## Performance

### Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing are performed to test for the presence of variants in coding regions and intron/exon boundaries of the *BTK* gene, as well as some other regions that have known disease-causing variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 30X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for deletions/insertions (delins) less than 40 base pairs (bp), and above 95% for deletions up to 75 bp and insertions up to 47 bp. NGS and/or a polymerase chain reaction-based quantitative method is performed to test for the presence of deletions and duplications in the *BTK* gene.

There may be regions of *BTK* that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences.(Unpublished Mayo method)

Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

Reference transcript numbers may be updated due to transcript re-versioning. Always refer to the final patient report for gene transcript information referenced at the time of testing.

| Gene symbol | Reference transcript | Additional evaluations | Technical limitations |
|---|---|---|---|
| *BTK* | NM_000061.2 | c.-193A>G<br>c.142-205A>G<br>c.240+108T>G<br>c.240+109C>A<br>c.895-11C>A<br>c.1102+2_1102+12del<br>c.1177+26_1177+27insGGTAGAAAAAA<br>c.1567-23A>C<br>c.1567-23A>G<br>c.1567-12_1567-9del | - |

### PDF Report
Supplemental

### Day(s) Performed
Varies

### Report Available
28 to 42 days

### Specimen Retention Time
Whole blood: 2 weeks (if available); Extracted DNA: 3 months; Cultured fibroblasts, skin biopsy: 1 month

### Performing Laboratory Location
Rochester

## Fees & Codes

### Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

### Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

### CPT Code Information
81406
88233- Tissue culture, skin, solid tissue biopsy (if appropriate)
88240- Cryopreservation (if appropriate)

## LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| BTKSG | BTK Gene, Full Gene Analysis | 94241-7 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 619761 | Test Description | 62364-5 |
| 619762 | Specimen | 31208-2 |
| 619763 | Source | 31208-2 |
| 619764 | Result Summary | 50397-9 |
| 619765 | Result | 82939-0 |
| 619766 | Interpretation | 69047-9 |
| 619767 | Additional Results | 82939-0 |
| 619768 | Resources | 99622-3 |
| 619769 | Additional Information | 48767-8 |
| 619770 | Method | 85069-3 |
| 619771 | Genes Analyzed | 82939-0 |
| 619772 | Disclaimer | 62364-5 |
| 619773 | Released By | 18771-6 |